

Appl. No. : **10/561,029**
Filed : **March 16, 2007**

REMARKS

The specification has been amended to provide the patent number corresponding to an application that was incorporated by reference. Claims 1 and 4 have been amended. New claims 31-34 have been added. Claims 20-29 have been withdrawn from consideration as being directed to a non-elected invention. Thus, claims 1 and 4-34 are now pending in the present application, with claims 1, 4-19 and 30-34 currently under consideration. Support for the amendment to claim 1 may be found in the specification at page 2, lines 12-25; and at col. 8, lines 21-34; column 15, lines 20-23 and column 16, lines 44-60 (Table 3) of US Patent No. 7,288,373, which is the patent that corresponds to Application No. 10/428,310 which was incorporated by reference in the present application at page 11, lines 7-8. Support for new claim 31 may be found in US Patent No. 7,288,373 at col. 7, lines 9-10. Support for new claim 32 may be found in US Patent No. 7,288,373 at col. 8, lines 35, 36 and 44-47. Support for new claim 33 may be found in US Patent No. 7,288,373 at col. 8, lines 36-37. Support for new claim 34 may be found in US Patent No. 7,288,373 at col. 8, lines 28-29. Thus, no new matter has been added. Reconsideration and withdrawal of the present rejections in view of the comments presented herein are respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1, 4-19 and 30 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. The Examiner stated that it was unclear how amplification could occur without including the primers of step (b) in the amplification step (c). Claim 1, step (c) as amended recites contacting the modified DNA and the population of random X-mers with nucleotides and a polymerase capable of amplifying double stranded DNA.

In view of the comments presented above, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 103(a)

Claims 1, 4, 5, 8-13 and 30 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Dean et al. (*Proc. Natl. Acad. Sci. USA* 99:5261-5266, 2002) in view of Berlin (US 7,008,770) and further in view of Olek (*Nucl. Acids Res.* 24:5064-5066, 1996) and further in view of Raizis et al. (*Anal. Biochem.* 226:161-166, 1995) ("combination 1").

Claims 6 and 7 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over combination 1, and further in view of Christensen et al. (US 2006/0014144).

Claims 14-19 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over combination 1, and further in view of Hogrefe et al. (US 2002/0143577).

Step (a) of claim 1 has been amended to recite that the treated genomic DNA is incubated at pH of between 10 and less than 12.5 after bisulphite treatment to form substantially unfragmented single stranded modified DNA, wherein cytosine bases but not 5'-methyl-cytosine bases in the genomic DNA are modified to uracil bases to form the modified DNA. This pH range is neither disclosed nor suggested by any of the references cited by the Examiner, either alone or in combination.

The present inventors have found that particular conditions in the desulfonation step, i.e. the step following bisulphite conversion, allows the integrity of the nucleic acid to be maintained. Claim 1 as amended incorporates some embodiments of these conditions, which unexpectedly result in minimal degradation of template DNA, resulting in substantially unfragmented single stranded modified DNA. Optimal desulfonation occurs in alkaline solutions. However, these conditions can also cause nucleic acid fragmentation, resulting in an assay with reduced sensitivity. Furthermore, incomplete desulfonation will result in nucleic acid that still contains sulphonated uracils, which DNA polymerases are unable to copy or bypass causing them to "drop off" the DNA strand, leading to a loss of PCR amplification products and further reducing the sensitivity of the assay. Consequently, the conditions recited in the present claims provide effective desulfonation without concomitant degradation of the nucleic acid.

The Examiner contends that Dean and Berlin provide the same methods for multiple displacement amplification for whole genome amplification as presently claimed, and that these methods in combination with those of Olek and Raizis would lead to the present invention. However, the desulfonation pH conditions taught by Olek and Raizis are not within the presently claimed range, and the present inventors have found that desulfonation at a pH of 10 to less than about 12.5, as recited in present claim 1, provide unexpected benefits as discussed below.

Enclosed herewith is a copy of a Rule 132 Declaration of Dr. John R. Melki, a scientist at Human Genetic Signatures Pty Ltd., the assignee of the present application, that was submitted during prosecution of US Patent No. 7,288,373. This declaration clearly shows the unexpected

Appl. No. : 10/561,029
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results obtained when the desulfonation reaction is carried out at a pH of between 10 and less than 12.5 as recited in present claim 1 compared to the pH used by Raizis (pH 13) and Olek (pH 9) (Declaration, paragraphs 8-19, Exhibit B). At pH 13, no PCR product was obtained at 65, 70, 80, 85 or 95°C. At pH 9, very little product was obtained at 65, 70, 80 or 85°C, and no product was obtained at 95°C. In contrast, under the pH conditions recited in the present claims, significant amounts of PCR products were obtained (Exhibit B).

There are additional advantages of the claimed method over the cited references. The methods used in Olek are based on initial shearing or cleaving of the DNA to form minute fragments, followed by bisulphite treatment using very small quantities of sample and reagents, preferably maintained within a capillary, and rely on a specialized robot to be able to accurately pipette the small volumes required (column 11, line 62 – column 12, line 19). Thus the presently claimed methods are much more straightforward than the prior art, do not require equipment that is not readily available in a laboratory and are capable of maintaining the integrity of bisulphite converted DNA, such that very large amplification products (>20 kb) from as little as 10 cells can be seen at various loci throughout the converted genome.

However, as indicated above, the methods of Olek and Raizis would not be suitable to generate a non-degraded bisulphite converted genome as the starting material from which to apply the genome amplification methods. For example, without the bisulphite conversion of the starting DNA, it would not be possible to use this method for methylation analysis of a whole genome. Among the advantages of the presently claimed invention is its ability, through improved and selected treatment conditions, to generate an intact, converted genome, which can then be interrogated in its entirety. The unexpected properties of the presently claimed method are neither disclosed nor suggested by the cited references, and could not have been predicted by one of ordinary skill in the art. Thus, the claimed invention is not obvious over the combination of Dean, Berlin, Olek and Raizis.

In view of the comments presented above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a).

Appl. No. : **10/561,029**
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No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

Applicants submit that all claims are in condition for allowance. However, if minor matters remain, the Examiner is invited to contact the undersigned at the telephone number provided below.


Appl. No. : 10/561,029
Filed : March 16, 2007

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 3/8/10

By: 
Neil S. Bartfeld, Ph.D.
Registration No. 39,901
Agent of Record
Customer No. 20,995
(619) 235-8550

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